Mad1 Checks In On Chromosome Attachments

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For a eukaryotic cell to divide properly, its chromosomes must be accurately segregated. Failure to correctly segregate chromosomes leads to aneuploidy (an incorrect number of chromosomes in the cell), which is a feature of many cancers as well as birth defects (Siegel and Amon, 2012). For chromosome segregation to occur, microtubules projecting from both mitotic spindles must attach to each chromosome. A specialized chromosomal region called the centromere serves as a platform for the assembly of a massive protein complex known as the kinetochore to which spindle microtubules attach. The kinetochore also mediates the spindle checkpoint, a conserved signal transduction cascade that prevents cell division until all chromosomes are properly attached to microtubules. "It has been a longstanding question how microtubule binding to the kinetochore is related to kinase signaling of the checkpoint," says graduate student Nitobe London. In a recent study, London in the laboratory of Dr. Sue Biggins (Basic Sciences Division), took advantage of a method to isolate kinetochore complexes from budding yeast cells to describe a new phosphorylation event that is crucial for spindle checkpoint activation.

The researchers first asked how Mad1, a protein important for spindle checkpoint activation, is recruited to kinetochores. To activate the spindle checkpoint, yeast cells were treated with benomyl, a drug that destabilizes microtubules. Mad1 associated with the kinetochore upon benomyl treatment. Mad1-containing protein complexes were isolated to identify binding partners. London found that a kinetochore protein called Spc105 was required for Mad1 binding to the kinetochore. This result is consistent with previous work showing that cells with compromised Spc105 function lack a functional spindle checkpoint.

To further delineate the mechanism by which Mad1 is recruited to kinetochores, the researchers focused on the Bub1/3 complex. Bub1/3 is required for Mad1 kinetochore recruitment and interacts with the kinetochore via Spc105. When the central region of Bub1, which is necessary for spindle checkpoint function in human cells, was deleted, Mad1 failed to associate with kinetochores. Notably, the central region of Bub1 was sufficient to recruit Mad1 to the kinetochore when it was fused to a mutant Spc105 protein that lacked several key phosphorylation sites.

The researchers next tested whether phosphorylation was generally required for Mad1 kinetochore

localization. Treatment of purified kinetochores with a phosphatase released Mad1, implicating a requirement for phosphorylation in Mad1 kinetochore binding. By mass spectrometry, several phosphorylation sites within the central region of Bub1 were identified, and simultaneous mutation of all phosphorylation sites compromised the interaction of Mad1 with the kinetochore. The researchers identified Mps1, a kinase necessary for spindle checkpoint activation, as being responsible for Bub1 phosphorylation and subsequent Mad1 kinetochore recruitment.

These results not only provide a detailed view of how Mad1 is recruited to the kinetochore to mediate spindle checkpoint function, but may also have important implications for targeting the spindle checkpoint in cancer. “Because cancer cells are typically aneuploid, they require robust spindle checkpoint function to propagate. One therapeutic approach attenuates checkpoint function by inhibiting specific kinases required for checkpoint activation," says London. "Our work identifies a novel Mps1 phosphorylation event that has a direct role in activating the checkpoint. This could guide drug development efforts towards targeting the specific checkpoint activating step of Mps1 instead of the kinase itself, potentially improving specificity."

London N, Biggins S. 2014. Mad1 kinetochore recruitment by Mps1-mediated phosphorylation of Bub1 signals the spindle checkpoint. *Genes Dev* 28(2):140-152.

See also: Siegel JJ, Amon A. 2012. New Insights into the Troubles of Aneuploidy. *Annu Rev Cell Dev Biol* 28:189-214.

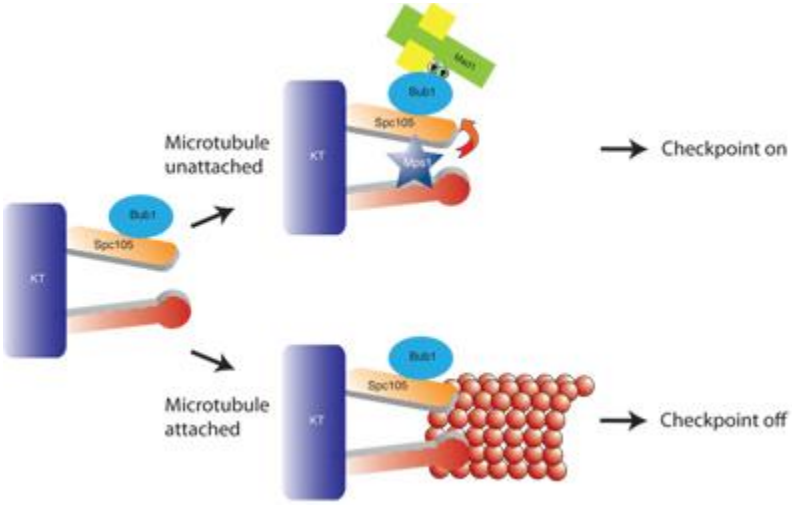

Image provided by Nitobe London

Model for the regulation of the spindle checkpoint. Spc105 (orange) and Bub1 (blue) are associated with the kinetochore (purple rectangle). If a microtubule is properly attached to the kinetochore, the checkpoint is not activated (bottom right). In the absence of proper microtubule attachment (top right), Mps1 (purple star) phosphorylates the middle region of Bub1, recruiting Mad1 to activate the spindle checkpoint, halting cell cycle progression.